REMARKS

The application has been amended to incorporate the SEQ ID NOS of the various sequences disclosed therein by their respective SEQ ID NOS as assigned in the Sequence Listing being submitted concurrently herewith. No new matter is introduced by virtue of these amendments. Accordingly, Applicants kindly request that they be entered into the instant application.

No fees are believed due in connection with this response. However, the Commissioner is authorized to charge all required fees, fees under 37 C.F.R. § 1.17 and all required extension of time fees, or credit any overpayment, to Pennie & Edmonds U.S. Deposit Account No. 16-1150 (order no. 10342-012-999).

Respectfully submitted,

Dated: August 22, 2001

42,983
Rahul Pathak (Reg. No.)

For: Laura A. Coruzzi (Reg. No.: 30,742)
PENNIE & EDMONDS LLP
1155 Avenue of the Americas
New York, New York 10036-2711
(212) 790-9090

Enclosures

Exhibit A

Marked Up Versions of Amended Paragraphs:

(Additions are underlined, deletions are bracketed)

On page 5, please replace the paragraph beginning, "FIG. 1 provides a structure-based sequence alignment of LuxS proteins..." with the following paragraph:

FIG. 1 provides a structure-based sequence alignment of LuxS proteins, H. pylori LuxS (SEQ ID NO: 1), D. radiodurans LuxS (SEQ ID NO: 3), H. inluenzae LuxS (SEQ ID NO: 2), C. Jejuni LuxS (SEQ ID NO: 4), B. burgdorferi LuxS (SEQ ID NO: 5), C. perfringens LuxS (SEQ ID NO: 6), N. meningitidis LuxS (SEQ ID NO: 7), S. typhimurium LuxS (SEQ ID NO: 8), V. harveyi LuxS (SEQ ID NO: 9), E.coli LuxS (SEQ ID NO: 10), V. cholerae LuxS(SEQ ID NO: 11), B. subtilis LuxS (SEQ ID NO: 12), and B. halodurans LuxS (SEQ ID NO: 13).

On page 32, please replace the paragraph beginning, "For native crystals from which the atomic structure coordinates of the invention are obtained, it has been found that sitting drops comprising about 1 µL of *H. pylori* LuxS polypeptide (5 mg/mL in 10 mM HEPES, pH 7.5, 150 mM sodium chloride..."with the following paragraph:

For native crystals from which the atomic structure coordinates of the invention are obtained, it has been found that sitting drops comprising about 1 µL of *H. pylori* LuxS polypeptide (SEQ ID NO: 1) (5 mg/mL in 10 mM HEPES, pH 7.5, 150 mM sodium chloride, 10 mM methionine, 1 mM beta-mercaptoethanol) with 1 µL reservoir solution (32% w/v PEG 1000, 200 mM ammonium sulfate, and 100 mM MES, pH 5.75) suspended over 0.5 mL reservoir solution for about one week at 20°C provide diffraction quality crystals. Similarly, sitting drops prepared by mixing about 1 µL of *D. radiodurans* LuxS polypeptide (SEQ ID NO:3) (19 mg/mL in 10 mM HEPES, pH 7.5, 150 mM sodium chloride, 10 mM methionine, 1 mM beta-mercaptoethanol) and 1 µL reservoir solution (26% w/v PEG monomethyl ether ("PEG MME") 5000, and 100 mM MES, pH 6.5) suspended over 0.5 mL reservoir solution for about one week at 4°C provide diffraction quality crystals. Sitting drops prepared by mixing about 1 µL of *H. influenzae* LuxS

polypeptide <u>(SEQ ID NO: 2)</u> (10 mg/mL in 10 mM HEPES, pH 7.5, 150 mM sodium chloride, 10 mM methionine, 1 mM beta-mercaptoethanol) and 1 μL reservoir solution (21% w/v PEG MME 5000, and 100 mM Bis-Tris, pH 6.25) suspended over 0.5 mL reservoir solution for about one week at 12°C provide diffraction quality crystals.

On page 36, please replace the paragraph beginning, "LuxS crystals were also obtained from *H. influenzae* LuxS. The *H. influenzae* LuxS crystals, which may be native crystals..."with the following paragraph:

LuxS crystals were also obtained from *H. influenzae* LuxS <u>(SEQ ID NO: 2)</u>. The *H. influenzae* LuxS crystals, which may be native crystals, heavy-atom derivative crystals or co-crystals, have a tetragonal unit cell and space group symmetry $P4_22_12$. In one form of crystalline *H. influenzae* LuxS, the unit cell has dimensions of a=129.59 +/- 1.3 Å, b=129.59 +/- 1.3 Å, c=53.74 +/- 0.5 Å. There are likely to be two LuxS molecules in the asymmetric unit, related by an approximate 2-fold axis. The crystals appear as long (up to 0.4 mm), thin (typically 0.05 to 0.1 mm wide) rods.

On page 36, please replace the paragraph beginning, "LuxS crystals of the invention were also obtained from *D. radiodurans* LuxS. In one form, the *D. radiodurans* LuxS crystals, which may be native crystals..."with the following paragraph:

LuxS crystals of the invention were also obtained from *D. radiodurans* LuxS <u>(SEQ ID NO:3)</u>. In one form, the *D. radiodurans* LuxS crystals, which may be native crystals, heavy-atom derivative crystals or co-crystals, have a monoclinic unit cell and space group symmetry $P2_1$. The unit cell has dimensions of a=43.71 +/- 0.4 Å, b=82.18 +/- 0.8 Å, c=49.48 +/- 0.5 Å and beta = 102.78 +/- 1.0 degrees. There are likely to be two LuxS molecules in the asymmetric unit, related by an approximate 2-fold axis. The crystals appear as small blocks (typically 0.05 to .1 mm on a side). In another form of *D. radiodurans* LuxS crystals, a C2 monoclinic unit cell is observed with dimensions of a=51.19 +/- 0.5 Å, b=70.14 +/- 0.7 Å, c=49.73 +/- 0.5 Å and beta = 112.03 +/- 1.1 degree. There is one molecule of LuxS in the asymmetric unit.

On page 40, please replace the paragraph beginning, "The present invention provides, for the first time, the high-resolution three-dimensional structure and atomic structure coordinates of crystalline LuxS as determined by X-ray crystallography..."with the following paragraph:

> The present invention provides, for the first time, the high-resolution three-dimensional structure and atomic structure coordinates of crystalline LuxS as determined by X-ray crystallography. The specific methods used to obtain the structure coordinates are provided in the examples, *infra*. The atomic structure coordinates of four crystalline forms of LuxS are appended as Table 7, Table 8, Table 9, and Table 10 (*H. pylori* <u>LuxS (SEQ ID NO: 1)</u>, *H. influenzae* <u>LuxS (SEQ ID NO: 2)</u>, *D. radiodurans* <u>LuxS</u> $P2_1$ <u>(SEQ ID NO: 3)</u>, and *D. radiodurans* <u>LuxS (SEQ ID NO: 3)</u> C2, respectively).

On page 41, please replace the paragraph beginning, "Examination of residual density after modeling of the LuxS protein revealed a patch of density near the metal binding site...."with the following paragraph:

> Examination of residual density after modeling of the LuxS protein revealed a patch of density near the metal binding site. This was successfully modeled to be a methionine (see FIG. 11). This ligand was seen in both molecules in the asymmetric unit for *H. pylori* <u>LuxS</u> <u>(SEQ ID NO: 1)</u> and *H. influenzae* <u>LuxS (SEQ ID NO: 2)</u> and in one of the molecules (B) in *D. radiodurans* <u>LuxS (SEQ ID NO: 3)</u>, space group $P2_1$. Methionine was present in the polypepide solution used for crystallization in 10mM concentration, added to keep Se atoms reduced. Thus there is no indication that methionine plays an *in vivo* role as a substrate for LuxS. Indeed, its sidechain is too short to reach the metal site (see FIG. 11). However, through modeling with the program SPOCK (Christopher, 1998, Texas A & M University) it is apparent that there is considerable room for a larger amino acid to bind in this region. When S-ribosylhomocysteine, a proposed substrate for LuxS (PCT WO 00/32152), was modeled into the amino acid binding site several highly conserved residues of LuxS were identified as significant due to their closed proximity to this ligand: Ser 9, His 14, Arg 23, Asp 40, Arg 42, Glu 60, Met 84, Cyc 86, Thr 88, and Tyr 91.

On page 42, please replace the paragraph beginning, "Three crystalline LuxS polypeptides displayed a homodimer interaction in their asymmetric units, and the fourth, the *D. radiodurans* C2 crystalline polypeptide...."with the following paragraph:

Three crystalline LuxS polypeptides displayed a homodimer interaction in their asymmetric units, and the fourth, the *D. radiodurans* C2 crystalline polypeptide (SEQ ID NO:3), displayed a dimer with crystallographic symmetry. The dimerization is illustrated in FIG. 7. This dimerization was highly consistent between the three structures (alpha carbon superpositions of the dimers ranging from 1.0 Å^2 for *D. radiodurans* LuxS (SEQ ID NO: 3) P2_1 onto *H. influenzae* LuxS (SEQ ID NO: 2) to 1.2 Å^2 for *D. radiodurans* LuxS (SEQ ID NO: 3) P2_1 onto *H. pylori* LuxS (SEQ ID NO: 1) for residues 11-69, 77-118, and 125-152 of each monomer). The surface area buried through this interaction is 3930 Å^2 for the *D. radiodurans* P2_1 LuxS, 3,195 Å^2 for the *D. radiodurans* LuxS (SEQ ID NO: 3) C2 crystallographic dimer, 4160 Å^2 for *H. influenzae* LuxS (SEQ ID NO: 2), and 4180 Å^2 for *H. pylori* LuxS (SEQ ID NO: 1). These are very significant, comprising nearly a quarter of each molecules surface. Thus we propose that LuxS functions as a homodimer in solution. More evidence for this is the fact that the methionine ligand binding occurs at the dimer interface (see FIG. 7B) and a channel is provided for ligand entrance and exit through the opposing molecule (see FIG. 12).

On page 52, please replace the paragraph beginning, "The subsections below describe the production of a polypeptide containing the *H. pylori* LuxS protein...."with the following paragraph:

"The subsections below describe the production of a polypeptide containing the *H. pylori* LuxS protein (SEQ ID NO: 1), and the preparation and characterization of diffraction quality crystals, heavy-atom derivative crystals."

On page 54, please replace the paragraph beginning, "The stereochemical quality of the atomic model was monitored using PROCHECK (Laskowski *et al.*, 1993, "PROCHECK: a program to check the stereochemical quality of LuxS structures...."with the following paragraph:

The stereochemical quality of the atomic model was monitored using PROCHECK (Laskowski *et al.*, 1993, "PROCHECK: a program to check the stereochemical quality of LuxS structures," J. Appl. Cryst. 26:283-291). As defined in PROCHECK, for *H. pylori* LuxS (SEQ ID NO: 1), there are 87.7% (molecule A) and 86.2% (molecule B) of the residues in the model have main-chain torsion angles in the most favored Ramachandran regions . No residues fall in the disallowed region. In H. pylori, there are only two residues of molecule A and none of molecule B that fall in the generously allowed regions. The overall G-factor scores are 0.16 (*H. pylori*, molecule A) and 0.13 (*H. pylori*, molecule B).

On page 54, please replace the paragraph beginning, "The subsections below describe the production of a polypeptide containing the *H. influenzae* LuxS protein...."with the following paragraph:

> The subsections below describe the production of a polypeptide containing the *H. influenzae* LuxS protein (SEQ ID NO: 2), and the preparation and characterization of diffraction quality crystals, heavy-atom derivative crystals.

On page 55, please replace the paragraph beginning, "The MAD data was indexed and intergrated using the program Denzo and merged and scaled using the program Scalepack. The program SnB was then used to determine the location of Selenium-methionine ..."with the following paragraph:

> The MAD data was indexed and intergrated using the program Denzo and merged and scaled using the program Scalepack. The program SnB was then used to determine the location of Selenium-methionine Se's based on the peak wavelength data. These Se sites (12 of 14 were found) were refined and phase information for the protein obtained using the program SHARP. Solomon solvent flattening of the data was subsequently employed in SHARP. The resulting map was viewed in the program O and found to be of excellent quality with cssentially all of both of the proteins in the asymmetric unit, main chain and sidechains, easily visible. This map was modeled using O to give the position of nearly all of thc residues: residues 6 through 164 (molecule A) and 6 through 166 (molecule B) of *H. influenzae* LuxS (SEQ ID NO: 2). The model was refined using thc program CNX.

On page 56, please replace the paragraph beginning, "The stercochemical quality of the atomic model was monitored using PROCHECK (Laskowski *et al.*, 1993, "PROCHECK: a program to check the stereochemical quality of LuxS structures ..."with the following paragraph:

The stereochemical quality of the atomic model was monitored using PROCHECK (Laskowski *et al.*, 1993, "PROCHECK: a program to check the stereochemical quality of LuxS structures," J. Appl. Cryst. 26:283-291). As defined in PROCHECK, for *H. influenzae* LuxS <u>(SEQ ID NO: 2)</u> there are 92.8% (molecule A) and 90.8% (molecule B) of the residues in the model have main-chain torsion angles in the most favored Ramachandran regions . There is only one residue that falls in the disallowed region (H. Influenzae, molecule B). H. influenzae has one residue of each molecule falling in the generously allowed regions. The overall G-factor scores are 0.25 (*H. influenzae*, molecules A and B).

On page 58, please replace the paragraph beginning, "The subsections below describe the production of a polypeptide containing the *D. radiodurans* LuxS protein ..."with the following paragraph:

The subsections below describe the production of a polypeptide containing the *D. radiodurans* LuxS protein <u>(SEQ ID NO:3)</u>, and the preparation and characterization of diffraction quality crystals, heavy-atom derivative crystals.

Exhibit B

MARKED UP VERSION OF AMENDED CLAIMS

2. (Amended) The crystal of Claim 1 wherein the LuxS is *H. pylori* LuxS (SEQ ID NO: 1), *H. influenzae* LuxS (SEQ ID NO: 2) or *D. radiodurans* LuxS (SEQ ID NO:3).

24. (Amended) The method of Claim 23 wherein the LuxS polypeptide is *H. pylori* LuxS polypeptide (SEQ ID NO: 1), *H. influenzae* LuxS polypeptide (SEQ ID NO: 2) or *D. radiodurans* LuxS polypeptide (SEQ ID NO:3).

33. (Amended) The machine readable medium of Claim 32, in which the LuxS is *H. pylori* LuxS (SEQ ID NO: 1), *H. influenzae* LuxS (SEQ ID NO: 2) or *D. radiodurans* LuxS (SEQ ID NO:3).

46. (Amended) The method of Claim 43 in which LuxS is *H. pylori* LuxS (SEQ ID NO: 1), *H. influenzae* LuxS (SEQ ID NO: 2) or *D. radiodurans* LuxS (SEQ ID NO:3).

51. (Amended) The method of Claim 48 in which the LuxS is *H. pylori* LuxS (SEQ ID NO: 1), *H. influenzae* LuxS (SEQ ID NO: 2) or *D. radiodurans* LuxS (SEQ ID3).

Exhibit C

PENDING CLAIMS AFTER ENTRY OF INSTANT AMENDMENT

1. A crystal comprising LuxS in crystalline form.

2. (Amended) The crystal of Claim 1 wherein the LuxS is *H. pylori* LuxS (SEQ ID NO: 1), , *H. influenzae* LuxS (SEQ ID NO: 2) or *D. radiodurans* LuxS (SEQ ID NO:3).

3. The crystal of Claim 1 which is diffraction quality.

4. The crystal of Claim 1 which is a native crystal.

5. The crystal of Claim 1 which is a heavy-atom derivative crystal.

6. The crystal of Claim 1 in which LuxS is a mutant.

7. The crystal of Claim 6, in which the mutant is a selenomethionine or selenocysteine mutant.

8. The crystal of Claim 6, in which the mutant is a conservative mutant.

9. The crystal of Claim 6, in which the mutant is a truncated or extended mutant.

10. The crystal of Claim 1 which is characterized by a diffraction pattern that is substantially similar to the diffraction pattern of FIG. 2., FIG 3., FIG 4. or FIG 5.

11. The crystal of Claim 1, which is characterized by a unit cell of a=71.04±0.7Å, b=71.04±0.7Å, c=130.14±1.3Å, a =90.0, b =90.0, and g =90.0.

12. The crystal of Claim 1, which is characterized by a unit cell of a=129.59±1.3Å, b=129.59±1.3Å, c=53.74±0.5Å, a =90.0, b =90.0, and g =90.0.

13. The crystal of Claim 1, which is characterized by a unit cell of a=43.53±0.5Å, b=81.87±0.8Å, c=49.30±0.5Å, a =90.0, b =102.85, and g =90.0.

14. The crystal of Claim 1, which is characterized by a unit cell of a=51.08±0.5Å, b=70.04±0.7Å, c=49.75±0.5Å, a =90.0, b =102.85, and g =90.0.

15. The crystal of Claim 1, which is produced by a method comprising the steps of:

(a) mixing a volume of a solution comprising the LuxS with a volume of a reservoir solution comprising a precipitant; and

(b) incubating the mixture obtained in step (a) over the reservoir solution in a closed container, under conditions suitable for crystallization until the crystal forms.

16. The crystals of Claims 11-14, wherein the precipitant is present in a concentration between about 15% and about 35% (w/v).

17. The crystals of Claims 11-14 wherein the precipitant is polyethylene glycol or PEG MME with an average molecular weight between about 1000 Da and about 10000 Da.

18. The crystals of Claims 11-14, wherein the solution further comprises between about 10 mM and about 200 mM buffer.

19. The crystals of Claim 18 wherein the buffer is HEPES, Tris, MES, MOPS, Bis-Tris, Sodium cacodylate, ACES, ADA, BES, or Citric acid.

20. The crystals of Claims 11-14, wherein the solution further comprises between 0 mM and about 300 mM ammonium sulfate.

21. The crystals of Claims 11-14, wherein the solution has a pH of between about 5.0 and about 7.0.

22. The crystals of Claims 11-14, which is produced by incubating the mixture comprising LuxS and reservoir solution at a temperature of between about 4 °C and about 25°C.

23. A method of making the crystal of Claim 1, comprising:

(a) mixing a volume of a solution comprising a LuxS polypeptide with a volume of a rescrvoir solution comprising a precipitant; and

(b) incubating the mixture obtained in step (a) over the reservoir solution in a closed container, under conditions suitable for crystallization until the crystal forms.

24. (Amended) The method of Claim 23 wherein the LuxS polypeptide is *H. pylori* LuxS polypeptide (SEQ ID NO: 1), *H. influenzae* LuxS polypeptide (SEQ ID NO: 2) or *D. radiodurans* LuxS polypeptide (SEQ ID NO:3).

25. The method of Claim 23, wherein the precipitant is PEG or PEG MME with an average molecular weight between about 1000 and about 10000.

26. The method of Claim 23, wherein the precipitant is present in a concentration between about 15 % and about 35 % (w/v).

27. The method of Claim 23, wherein the solution further comprises between about 10 mM to about 200 mM buffer.

28. The method of Claim 27 wherein the buffer is HEPES, Tris, MES, MOPS, Bis-Tris, Sodium cacodylate, ACES, ADA, BES, or Citric acid.

29. The method of Claim 23, wherein the solution further comprises between about 0 mM and about 300 mM ammonium sulfate.

30. The method of Claim 23, wherein the solution has a pH of between about 5.0 and about 7.0.

31. The method of Claim 23, wherein the mixture comprising LuxS and reservoir solution is incubated at a temperature of between about 4 °C and about 25 °C.

32. A machine-readable medium embedded with information that corresponds to a three-dimensional structural representation of a crystal comprising LuxS in crystalline form, or a fragment or portion thereof.

33. (Amended) The machine readable medium of Claim 32, in which the LuxS is *H. pylori* LuxS (SEQ ID NO: 1), *H. influenzae* LuxS (SEQ ID NO: 2) or *D. radiodurans* LuxS (SEQ ID NO:3).

34. The machine readable medium of Claim 32, in which thc crystal is diffraction quality.

35. The machine readable medium of Claim 32, in which the crystal is a native crystal.

36. The machine readable medium of Claim 32, in which the crystal is a heavy-atom derivative crystal.

37. The machine readable medium of Claim 32, in which the crystalline LuxS is a mutant.

38. The machine readable medium of Claim 37, in which the mutant is a selenomethionine or selenocysteine mutant.

39. The machine readable medium of Claim 37, in which the mutant is a conservative mutant.

40. The machine readable medium of Claim 37, in which the mutant is a truncated or extended mutant.

41. The machine-readable medium of Claim 32, in which the information comprises the atomic structure coordinates, or a subset thereof.

42. A machine-readable medium embedded with the atomic structure coordinates of

Table 7, Table 8, Table 9, or Table 10, or a subset thereof.

43. A method of identifying a LuxS binding compound, comprising the step of using a threc-dimensional structural representation of LuxS, or a fragment thereof comprising

a LuxS substrate binding site, to computationally screen a candidate compound for an ability to bind the LuxS substrate binding site.

44. The method of Claim 43 further including the steps of:

synthesizing the candidate compound; and

screening the candidate compound for LuxS binding activity.

45. The method of Claim 43 in which the structural information comprises the atomic structure coordinates of residues comprising a LuxS substrate binding site.

46. (Amended) The method of Claim 43 in which LuxS is *H. pylori* LuxS (SEQ ID NO: 1), *H. influenzae* LuxS (SEQ ID NO: 2) or *D. radiodurans* LuxS (SEQ ID NO: 3).

47. A method of identifying a LuxS binding compound comprising the step of using a three-dimensional structural representation of LuxS, or a fragment thereof comprising a LuxS substrate binding site, to computationally design a synthesizable candidate compound that binds LuxS.

48. The method of Claim 47 in which the computational design comprises the steps of:

identifying chemical entities or fragments capable of associating with the LuxS substrate binding site; and

assembling the chemical entities or fragments into a single molecule to provide the structure of the candidate compound.

49. The method of Claim 48 further including the steps of:

synthesizing the candidate compound; and

screening the candidate compound for LuxS binding activity.

50. The method of Claim 48 in which the structural information comprises the atomic structure coordinates of residues comprising a LuxS substrate binding site.

51. (Amended) The method of Claim 48 in which the LuxS is *H. pylori* LuxS (SEQ ID NO: 1), *H. influenzae* LuxS (SEQ ID NO: 2) or *D. radiodurans* LuxS (SEQ ID NO: 3).

52. A method of designing a mutant LuxS comprising the steps of:

identifying a functional amino acid residue in the primary sequence of a three-dimensional representation of a LuxS molecule produced with the machine readable medium of Claim 32; and

altering the functional amino acid residue in the primary sequence of the LuxS molecule.

53. A method of preparing a mutant LuxS comprising:

desinging a mutant LuxS according to Claim 52; and

synthesizing the mutant LuxS.